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Augmented breath phase volume and timing relationships in the anesthetized rat

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Abstract

Augmented breaths (ABs), or sighs, are airway protective reflexes and part of the normal repertoire of respiratory behaviors. ABs consist of two phases, where phase I volume and timing resembles preceding eupnic breaths, and phase II is an augmenting motor pattern and occurs at the end of phase I. Recent evidence suggest multiple respiratory motor patterns can occur following dynamic functional reconfiguration of one respiratory neural network. It follows that the response of the respiratory network to modulatory inputs also may undergo dynamic reconfiguration. We hypothesized that lung-volume related feedback during ABs would alter AB timing differentially during phase I and II. We measured phase I and II volumes and durations in urethane anesthetized rats with decreased lung volume secondary to three models of varying phrenic motor impairment (spinal injury alone, unilateral phrenicotomy, and combined injuries). AB phase I and II inspired volume were decreased after phrenic motor impairment ($p < 0.05$). In contrast, only phase I duration following injury was altered compared to controls. Phase II duration remaining unchanged despite the greatest effect of injury on volume occurring during phase II. Thus, sigh volume–timing relationships differ between phases of an augmented breath suggesting that the response of the respiratory network to modulatory inputs has changed. These data support the hypothesis that multiple respiratory behaviors occur following dynamic reconfiguration of the respiratory neural network.

Keywords

Phrenic; Sighs; Breathing

Sighs, or augmented breaths (ABs), occur episodically and their physiological benefit has been attributed to expansion of atelectic areas of lung restoring lower airway patency and compliance [11]. Recent evidence suggests that ABs, like coughs [3], might represent dynamic functional reconfiguration of one brainstem rhythmogenic network, the same network responsible for eupnea [9]. Evidence from invertebrates [10] makes it plausible that the response of the respiratory network to modulatory inputs also may undergo dynamic reconfiguration. For example, eupnic breathing is characterized by a vagally mediated volume–timing relationship that is hyperbolic where increasing inspiratory volume is associated with decreasing inspiratory duration [5]. In contrast, cough is associated with large increases in inspiratory volume and no change in duration, suggesting an absence of pattern modulation by lung volume-related feedback during this respiratory behavior [2].

The relationship between vagally mediated lung-volume feedback and AB duration is less clear. ABs are comprised of two distinct inspiratory phases (I and II) [4,9]. Phase I volume and timing resembles the preceding eupnic breaths, while phase II is an augmenting motor pattern and occurs at the end of phase I (see Fig. 1). In vagally intact cats, hypoxia can increase AB inspiratory volume without altering phase duration suggesting that some aspects of this reflex may be stereotypical and not subject to the volume–timing relationship observed during eupnea [4]. We chose to examine AB volume–timing relationships in animals with low lung volume to avoid chemoreceptor stimulation and the profound effects those reflexes have on the control of breathing. We hypothesized that decreased lung volume would decrease AB volume without altering AB phase duration. Low lung volumes were induced by various phrenic motor impairment models: incomplete high cervical spinal cord injury (C₂ hemi-section), unilateral phrenicotomy, and combined C₂ hemi-section with ipsilateral phrenicotomy. The dual injury group was included because modest motor recovery develops in the previously quiescent phrenic nerve by 2-month post-spinal injury and contributes to tidal volume [8].

Forty-eight specific pathogen free female rats (Harlan Sprague Dawley, Indianapolis, IN, USA) ranging in mass from 225 to 316 g were used in this study. Animals were divided into an uninjured group and three models of phrenic motor impairment with their sham-operated controls. The three specific injury types were chosen because the magnitude of phrenic motor impairment, and lung volume, varies between models [8]. Animals were allocated to normal ($n = 6$), C₂ hemisection ($n = 9$), C₂ hemisection sham-operated ($n = 6$), unilateral phrenicotomy ($n = 7$), thoracotomy-only ($n = 6$), C₂ hemisection and thoracotomy ($n = 6$), and combined C₂ hemisection and phrenicotomy on the side of C₂ injury ($n = 8$) groups. Animals were subsequently evaluated at 2-month post-injury. Animal husbandry and all procedures were in compliance with the Institutional Animal Care and Use Committee at the University of Florida, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Rats received surgically induced spinal cord injuries while anesthetized. Rats were initially anesthetized with medetomidine (75 µg/kg i.m.) and isoflurane in oxygen delivered via nose cone. After oro-tracheal intubation, anesthesia was maintained with isoflurane in oxygen and rats were mechanically ventilated. A laminectomy was made at the 2nd cervical vertebral level and the C₂ spinal segment and the cranial segment of C₃ were exposed. A 1-mm-long left-sided hemisection was made in C₂ and the excised tissue aspirated with a fine tipped glass pipette. All animals were allowed to recover and received atipamezole (0.1 mg/kg i.v.) to antagonize the anesthetic effects of medetomidine. Buprenorphine (50 µg/kg i.v.) and carprofen (5 mg/kg i.v.) were administered for post-surgical pain control. Analgesics were repeated as required over the next 2 days. In rats that were sham-operated, the procedure was the same but the spinal cord was left intact.

Phrenicotomy surgeries were performed under similar anesthetic conditions. A 1.5-cm-long left lateral thoracotomy was made between the 8th and 9th rib adjacent to the costochondral junction. Elevating the left caudal lung lobe allowed the left phrenic nerve to be visualized running in a craniocaudal direction adjacent to the caudal vena cava. A 1.2–1.5-cm segment of the phrenic nerve was removed using a surgical hook and micro-scissors. Phrenicotomy occurred prior to the nerve arborizing to supply the diaphragm. Lidocaine (4 mg/kg total dose, 2% solution) was administered via injection into the intercostal muscles dorsal to the thoracotomy incision for post-operative analgesia. Closure of the wound was achieved by using standard suturing techniques. Post-surgical drug administration was similar to above. For the sham-operated group, the procedure was the same but the phrenic nerve was left intact after elevating the left caudal lung lobe.

At 2-month post-surgery, rats were anesthetized with urethane (1.5 g/kg i.p.). Anesthesia was maintained by administering urethane (0.2–0.3 g/kg i.v.) as needed. A catheter was placed in a femoral artery to allow monitoring of direct arterial blood pressure and to allow collection of arterial blood for blood gas analysis (iSTAT, Waukesha, WI, USA). Each blood gas measurement required 0.15 ml of blood from each rat.

A femoral vein catheter was placed to administer drugs and fluids. Atropine sulfate (0.2 mg/kg i.v.) was administered to decrease upper respiratory secretions. The trachea was cannulated at the mid-cervical level and rats were allowed to breathe spontaneously on room air throughout the study. Rectal temperature was maintained at 38.0 ± 0.5 °C with an electric heating pad. All blood gas measurements were corrected to the rectal temperature at the time of sampling. When surgical preparation was completed, rats were placed in a supine position and a pneumotachometer (Hans Rudolf Inc., Kansas City, MO, USA) was attached to the tracheal cannula. The pneumotachometer was calibrated prior to, and at intervals during, the study using a square wave pulse of known volume and duration. Airflow was recorded using a differential pressure transducer (model MP45-14-871, Validyne, Northridge, CA, USA) attached to the pneumotachometer. Airflow was electrically integrated to derive volume. Each breath phase (inspiration and expiration) was integrated separately providing continuous display of both inspiratory volume (V_I) and expiratory volume (V_E).

After the pneumotachometer was attached to the tracheotomy tube, rats were allowed to breathe for 30 min before a 10-min period of airflow recording was obtained and represented spontaneous ventilation. Arterial blood was sampled at the end of the recording period. After the sample was taken, 0.4 ml of 0.9% saline was administered intravenously to replace lost blood volume.

Arterial blood pressure, inspiratory and expiratory airflow, and V_I and V_E were recorded on VCR tape, and digitized online by a computer based data analysis system (CED 1401, UK) and chart recorder. V_E , inspiratory time (T_I), and expiratory time (T_E), were averaged over five consecutive breaths immediately prior to blood gas analysis. V_E was measured as the peak integrated expiratory volume. T_I and T_E were measured from the airflow signal using zero airflow as the point of phase transition. Respiratory rate was calculated by dividing 60 s by ($T_I + T_E$ in seconds). Expired minute volume was calculated by multiplying expired volume by respiratory rate. Both V_E and minute volume were indexed to body weight.

All values are expressed as means $\pm$ S.E. Normality of the data was assessed using the Kolmogorov–Smirnov test and equivalency of variance was assessed using Bartlett's test. Nonparametric means were compared using the Kruskal–Wallis ANOVA followed by Mann–Whitney U -tests when indicated. All other means were compared using ANOVA for the factor “type of injury” (factor levels: control, C₂ hemisection, phrenicotomy, and combined C₂ hemisection and phrenicotomy). Multiple comparisons across groups were made using the Student–Newman–Keul's t -test. Paired means were compared using paired t -tests. Differences were considered significant if $p < 0.05$.

Normal, thoracotomy-only, and sham-operated groups were combined as one control group because no significant differences were identified between them. Similarly, C₂ hemisection and C₂ hemisection-thoracotomy groups were combined as one spinal injury group. The effects of C₂ hemisection with and without unilateral phrenicotomy on cardiovascular measurements, arterial blood gases, and eupnic-like breathing have been published elsewhere [8]. In brief, all models of phrenic motor impairment induced rapid shallow breathing compared to controls.

ABs were observed episodically during the 10-min recording period. An AB was identified based on the criteria of Cherniack et al. (1981): (1) a biphasic shape in which phase I

resembles the volume and duration of a preceding eupnic breath, (2) a distinct phase II with larger airflow and volume, and (3) lower volume of the eupnic breath immediately following an AB (see Fig. 1).

AB total volume (phase I+phase II volumes) decreased after injury due to reductions in both phase I and phase II volumes (Figs. 2 and 3; $p < 0.05$). Phase I volume and duration were quantitatively similar to preceding eupnic breaths (Table 1). The decrease in phase I volume following injury was accompanied by a decrease in phase I inspiratory duration (Table 1; Figs. 2 and 3; $p < 0.05$). In contrast, phase II duration was similar across all groups despite the effect of injury on volume being most pronounced during this phase (Figs. 2 and 3). No effect of injury was present on AB total inspiratory duration (Fig. 3) and expiratory duration (control: 0.23 ± 0.01 ; C_2 hemisection: 0.19 ± 0.01 ; phrenicotomy: 0.18 ± 0.01 ; combined C_2 hemisection and phrenicotomy: 0.22 ± 0.03).

In this study, we have demonstrated that reductions in AB volume during phase II are not associated with changes in phase II duration. Thus, the augmenting component of an AB is stereotypical and not subjected to the same volume-related feedback associated with eupnic breathing. Breathing comprises multiple behaviors: some generate tidal volume (i.e., eupnea), while others are airway protective (i.e., ABs and coughs). ABs may represent dynamic functional reconfiguration of the brainstem rhythmogenic network responsible for eupnea [9]. Dynamic motor networks also have been described in invertebrates where the response of network components to neuromodulatory inputs can also undergo dynamic changes [10]. Thus, it is plausible that the response of the respiratory network to modulatory inputs also may undergo dynamic reconfiguration (i.e., transient alterations in regulatory integration of peripheral afferent feedback). Cough, another airway protective reflex and which also may arise from network reconfiguration, does not follow the typical hyperbolic volume–timing relationship described for eupnic breathing [2]. Our results demonstrate that transient differences in inspiratory spiratory regulation occurs between multiple respiratory behaviors. Thus, our data supports the hypothesis that diverse respiratory motor patterns may occur following dynamic reconfiguration of the respiratory neural network.

The volume–timing relationship for eupnic breathing has been described as hyperbolic with increasing inspiratory volume associated with decreasing inspiratory phase duration [5]. During eupnea, volume and timing are regulated by slowly adapting receptor afferent feedback, which provides an “off-switch” terminating inspiration: as volume increases inspiration is terminated earlier, shortening inspiratory duration [6]. In our study, AB phase I (and eupnic breathing) volume and timing are decreased together. It is likely that, immediately following injury, reduced tidal volume is associated with increased T_i , however, in the long term, we believe a new steady-state is reached where these combinations of low volume and shortened inspiratory duration (and ultimately increased respiratory frequency) are required to maintain minute ventilation. The hyperbolic nature of the normal volume–timing relationship represents reflex responses to transient alterations in volume (i.e., via loading studies). Although not studied here, we believe a hyperbolic volume–timing relationship also would exist after injury, albeit with a left shift.

Previous studies have proposed that AB volume and phase duration may not be subjected to similar control [4,7]. For example, in cats, sigh total volume and duration are both larger than their preceding eupnic breaths and therefore do not follow the normal eupnic hyperbolic relationship [7]. In addition, Cherniack et al. (1981) demonstrated that hypoxia-induced augmentation of phase II volume in cats occurred without concurrent change in phase II duration. In the current study, we investigated the effects of decreasing phase I and II volume on their respective durations by impairing phrenic motor performance. The advantages of this approach were to avoid chemoreceptor stimulation, which can have

profound effects on respiratory timing. For example, unless rigidly controlled, changes in PaO_2 and PaCO_2 during hypoxic ventilation can have dramatic, yet transient, effects on inspiratory duration. We chose a “stimulus” (chronic phrenic motor impairment) to reduce volume that, by its nature, was at constant “intensity” during the experiment. Our approach also has the advantage of studying conditions that are typically associated with AB production in awake breathing animals. For each group of rats, phase I AB volume and duration were identical to preceding eupnic breaths. In contrast, decreased AB phase II volume after injury was not associated with changes in phase II duration. Thus, phase II duration appears stereotypical and regulated differently than phase I. Collectively, these results support conclusions made by earlier investigators that sighs are composed of an additional inspiratory effort on top of a “normal breath” [1,4,7], albeit differentially regulated. Recently, pharmacological methods were used to temporally separate each inspiratory phase further demonstrating sighs are composed of two distinct respiratory behaviors [9]. The mechanisms whereby the brainstem respiratory network is reconfigured to generate ABs is unknown.

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Fig 1.

Representative airflow and inspired volume traces from a control rat. The biphasic shape of the airflow and volume trace identifies an augmented breath. An augmented breath can be separated into phase I and phase II using the criteria of Cherniack et al. (1981). Phase I volume (V_{I1}) and duration (T_{I1}) and phase II volume (V_{I2}) and duration (T_{I2}) where measured after identifying the point of phase transition (vertical dashed line). I, inspiration; E, expiration; T_E , expiratory time.

Fig. 2. Augmented breaths from a control (CTL) and spinally injured (SCI) rat. Phase I and II volume and phase I duration decrease after injury, whereas phase II duration does not change.

Fig. 3.

Augmented phase volume s and durations from control (Ctl; $n = 18$), C_2 hemisection (SCI; $n = 15$), unilateral phrenicotomy (PX; $n = 7$), and combined injury (SCI/Px; $n = 8$) groups. Phase I volume (V_{I1}) and duration (T_{I1}), phase II volume (V_{I2}) and duration (T_{I2}), and total volume ($V_{I\text{total}}$) and duration ($T_{I\text{total}}$) were measured from each group. All injuries decreased V_{I1} , V_{I2} , and $V_{I\text{total}}$. In addition, V_{I2} and $V_{I\text{total}}$ were lower in the combined injury group compared to SCI. All injuries decreased T_{I1} compared to controls. No effects of injury on T_{I2} and $T_{I\text{total}}$ were present. Means $\pm$ S.E. * $p < 0.05$ relative to controls; † $p < 0.05$ relative to SCI.

Table 1

Comparison of preceding eupnic breath volume and timing with augmented breath phase I volume and timing

	Control	C ₂ hemisection	Phrenicotomy	Dual injuries
Eupnic V_I (ml/kg)	8.1 ± 0.2	6.3 ± 0.1 *	6.4 ± 0.3 *	5.9 ± 0.2 *
AB V_{I1} (ml/kg)	8.0 ± 0.1	6.5 ± 0.1 *	6.8 ± 0.2 *	6.4 ± 0.2 *
Eupnic T_I (s)	0.22 ± 0.02	0.16 ± 0.01 *	0.17 ± 0.01 *	0.16 ± 0.01 *
AB T_{I1} (s)	0.20 ± 0.01	0.17 ± 0.01 *	0.16 ± 0.01 *	0.15 ± 0.01 *

* $p < 0.05$, different to controls.